Progress in Biophysics and Molecular Biology xxx (2018) 1–12

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

Original Research

Multiparametric slice culture platform for the investigation of human cardiac tissue physiology

Yun Qiao, Quan Dong, Baichen Li, Sofian Obaid, Christian Miccile, Rose T. Yin, Trisha Talapatra, Zexu Lin, Sihui Li, Zhenyu Li, Igor R. Efimov*

Department of Biomedical Engineering, The George Washington University, Washington DC, 20052, USA

ARTICLE INFO

Article history:
Received 16 January 2018
Received in revised form
14 April 2018
Accepted 3 June 2018
Available online xxx

Keywords:
Cardiac electrophysiology
Optical mapping
Heart-on-a-chip
Tissue engineering
Drug development
Microfluidics

ABSTRACT

Human cardiac slices have emerged as a promising model of the human heart for scientific research and drug testing. Retaining the normal tissue architecture, a multi-cell type environment, and the native extracellular matrix, human cardiac slices faithfully replicate organ-level adult cardiac physiology. Previously, we demonstrated that human cardiac tissue slices cultured for 24 h maintained normal electrophysiology. In this project, we further optimized the organotypic culture condition to maintain normal electrophysiology of the human cardiac slices for 4 days. The prolonged culture of human cardiac tissue slices demonstrated here enables the study of chronic drug effects, gene therapies, and gene editing. To achieve greater control of the culture environment, we have also developed an automated, self-contained heart-on-a-chip system. The culture system supports media circulation, oxygenation, temperature control, electrical stimulation, and static mechanical loading. The culture parameters can be individually adjusted to establish the optimal culture condition to achieve long-term culture and to minimize tissue dedifferentiation. The development of the heart-on-a-chip technology presented here further encourages the use of organotypic human cardiac slices as a platform for pre-clinical drug testing and research in human cardiac physiology.

1. Introduction

Physiologically and genetically accurate models of the human heart are indispensable for studying human cardiac physiology and for pre-clinical screening of candidate biological and drug therapies for their efficacy and/or toxicity. Although crucial for fundamental biological discovery, animal models often fail to predict human response to treatments due to inter-species genetic and physiological differences (Hasenfuss, 1998; Mak et al., 2014; Nerbonne et al., 2001; The FANTOM Consortium and the RIKEN PMI and CLST (dgt), 2014). In recent years, human cardiac slices from donor and end-stage failing hearts have emerged as a promising model of the human heart for electrophysiological and pharmacological studies (Brandenburger et al., 2012; Camelliti et al., 2011). We have previously demonstrated that human cardiac slices faithfully recapitulate tissue level human cardiac physiology, exhibiting normal conduction velocity (CV), action potential duration (APD), intracellular calcium dynamics, heart rate dependence of these parameters and their response to α- and β-adrenergic stimulation (Kang et al., 2016).

Extensive efforts have been invested into developing an authentic model of the human heart. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) are widely used in modeling diseases and drug screening (Chi, 2013; Itzhaki et al., 2011). However, the development of hiPSC-CMs with mature atrial or ventricular phenotypes have been challenging so far (Karakikes et al., 2015; Robertson et al., 2013). Another approach to study human cardiac cell biology involves isolated primary human cardiomyocytes which can be used for high-throughput testing methods such as automatic patch clamping and optical mapping. Although these cells are functionally mature, they have limited experimental life-time since they dedifferentiate in cell culture (Bird et al., 2003; Coppini et al., 2014). Different cardiomyocyte subpopulations can be obtained by altering the cell isolation process. However, they exhibit altered electrophysiology, i.e. action potential morphology, due to the lack of cell-cell coupling and membrane protein alterations caused by the tissue digestion

* Corresponding author. Suite 5000, 800 22nd Street NW, Washington DC, 20052, USA.
E-mail address: efimov@gwu.edu (I.R. Efimov).

https://doi.org/10.1016/j.pbiomolbio.2018.06.001
0079-6107/

procedure. The cell isolation procedure is also time consuming and labor intensive, thus limiting the use of isolated cardiomyocytes to low-throughput testing. Another approach is based on the human ventricular wedge preparations, which allow for studying CV, conduction heterogeneity, and arrhythmia susceptibility (Glukhov et al., 2012, 2010a; Lou et al., 2011a). Due to the complexity and variability of the coronary system and the size constraint of the preparation, the ventricular wedge preparation is also severely limited in terms of throughput. Primary cells, cell lines, and tissue also have significantly different gene expression profiles, as reported by the functional annotation of the mammalian genome 5 consortium (The FANTOM Consortium and the RIKEN PMI and CLST (dgt), 2014). Human cardiac slices faithfully replicate the organ-level adult cardiac physiology because they retain the normal tissue architecture, a multi-cell type environment, the extracellular matrix, etc. (Kang et al., 2016). At the diffusion limit for oxygen, human cardiac slices can be cultured for extended period of time for studying chronic drug treatment, gene expression regulation, and genetic engineering (Barclay, 2005; Brandenburger et al., 2012; Bussek et al., 2012; Kang et al., 2016).

Previous studies on the organotypic culture of ventricular slices obtained from adult mammalian hearts mostly have been limited to 48 h, which diminishes the usefulness of the preparation for testing the effect of chronic drug and gene therapies (Brandenburger et al., 2012; Bussek et al., 2012; Kang et al., 2016). Lacking pacemaking abilities, slices collected from the ventricles of the heart undergo significant dedifferentiation when cultured with conventional tissue culture techniques that lack electrical or mechanical stimulation and loading (Brandenburger et al., 2012; Kaneko et al., 2012). Human tissue and primary cells have been implemented in numerous body-on-a-chip systems designed for drug testing (Esch et al., 2016; Loskill et al., 2017; Phan et al., 2017). However, due to difficulties in maintaining the mature phenotype of adult human cardiac tissue *in vitro*, thus far there has not been a heart-on-a-chip system that supports long-term organotypic culture of the human cardiac tissue. In this project, we present an improved conventional organotypic culture method that maintains normal electrophysiology of the human cardiac slices for 4 days. The prolonged culture of human cardiac tissue slices demonstrated here enables the study of chronic drug effects, gene therapies, and gene editing. To achieve greater control of the culture environment, we also have developed an automated, self-contained heart-on-a-chip system for organotypic culture of human cardiac tissue slices. The culture system supports media circulation, oxygenation, temperature control, electrical stimulation, and static mechanical loading. The system is also entirely self-contained to allow for the transport of live cardiac slices to share for scientific research and drug testing. The human heart-chip system provides a user-friendly platform for optimizing the slice culture condition to achieve long-term culture while maintaining adult cardiomyocyte phenotype.

2. Methods

2.1. Collection of human heart

Experimental protocols were approved by the George Washington University Institutional Review Board and were in accordance with human research guidelines. Various human donor hearts rejected for organ transplantation were procured from Washington Regional Transplant Service (Washington, DC). Consents were obtained from either donors as previously granted or from family members allowing use of the hearts for research purposes. Following aortic cross-clamp, the hearts were cardioplegically arrested using University of Wisconsin (UW) solution (ViaSpan) in the operating room. The hearts were transported in the UW solution on ice.

2.2. Slice preparation

The procedures for slice preparation and culturing were described previously in detail (Kang et al., 2016). A sample approximately 1 cm × 1 cm × 1 cm was cut from the left ventricular (LV) free wall. Care was taken to ensure that the tissue was submerged in solutions at all times. The tissue samples were mounted onto the tissue holder of a vibrating microtome (Campden Instruments, UK) and sectioned into 380 μm thick slices while submerged in a modified Tyrode's solution (140 mM NaCl, 6 mM KCl, 10 mM Glucose, 10 mM Hepes, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM BDM, pH 7.4). Tissue slices were collected at random depths in the mid-section of the lateral left ventricular free wall. The cutting chamber was surrounded by ice to maintain a stable low temperature. The vibrating microtome was set to 0.4 mm/s advance speed and 2 mm horizontal vibration amplitude at 80Hz. Importantly, to minimize damage to the tissue during sectioning, the unwanted vertical vibration of the blade was laser-calibrated to less than 0.5 μm. After sectioning, each slice was placed in a cell strainer, weighed down with a meshed ring, and transferred to a bath of modified Tyrode's washout solution (140 mM NaCl, 4.5 mM KCl, 10 mM Glucose, 10 mM Hepes, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, pH 7.4). The slices were kept in the washout solution for 20 min to wash out the BDM and reduce temperature shock before optical mapping or culturing.

2.3. Isolation of murine atrial preparation

Mice were anesthetized in accordance with animal protocol approved by The George Washington University IACUC. Following the loss of withdraw reflex, thoracotomy was performed to excise the heart, which was then Langendorff perfused with oxygenated Tyrode's solution (128.2 mM NaCl, 4.7 mM KCl, 11.1 mM Glucose, 1.3 mM $CaCl_2$, 1.05 mM $MgCl_2$, 1.19 mM NaH_2PO_4, 20 mM $NaHCO_3$) with pH adjusted to 7.4 at 37 °C. With the posterior side of the heart facing up, a cut was made slightly above the midsection of the heart to remove the ventricles. Facing the same orientation, an incision from the tricuspid valve to the superior vena cava along the atrial septum was made under a surgical microscope. Subsequently, a portion of the atrial septum was removed to open the left atria. The edges of the atria were slightly stretched and pinned with the endocardial surface up. The resulting isolated atrial preparation preserved the intact SAN region, delimited by the crista terminalis, atrial septum, and orifice of the superior vena cava.

2.4. Conventional culture of human cardiac slices in an incubator

To prevent contamination, the cardiac slices were rinsed three times in sterile phosphate buffered saline (PBS) before culturing. The forceps used to handle the slices were sterilized with a bead sterilizer before each rinse. The slice culture medium consisted of Medium 199 (M4530, Sigma-Aldrich, St. Louis, MO), 1x ITS (I3146, Sigma-Aldrich, St. Louis, MO), and 2% penicillin streptomycin (P4333, Sigma-Aldrich, St. Louis, MO). The slices were cultured in 6-well plates with one slice in each well and 3 mL of the culture medium. During culture, the slices were free-floating in the 6-well plates. To facilitate oxygen diffusion into the slices, the plates were agitated on an orbital shaker at 20 rpm placed inside a tri-gas incubator (Thermo Fisher Scientific, Waltham, MA) with 30% O_2 and 5% CO_2 at 37 °C. The culture medium was changed every two days.

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

2.5. Optical mapping of mouse atrial tissue and human cardiac slices

A CMOS camera imaging system (ULTIMA-L, SciMedia, Costa Mesa, CA) was used to measure changes in transmembrane potential in the acutely isolated and cultured murine atria and human slices. Both types of tissue were superfused in Tyrode's washout solution at 37°C with the pH maintained at 7.4 in a temperature controlled tissue bath. To eliminate motion artifacts in the recorded optical signal, the human cardiac tissue slices and isolated atrial tissue were immobilized using the excitation-contraction uncoupling agent, blebbistatin (13186, Cayman Chemical, Ann Arbor, MI). Prior to performing optical mapping, we slowly dispensed 200 μL of 10 μM Blebbistatin on top of the tissue and allowed to incubate for 5 min. Subsequently, 500 μL of 5 μM Di-4-ANEPPS (61010, Biotium, Fremont, CA), a voltage-sensitive dye, was loaded onto the tissue in a similar manner. A green LED light source (Prizmatix, Southfield, MI) with the wavelengths of 520 ± 45 nm was used to excite the voltage-sensitive dye. The emitted fluorescence was filtered by a long-pass filter at 650 nm and collected by the ULTIMA-L camera as previously described (Kang et al., 2016).

2.6. Optical mapping data analysis

The recorded data was first visualized using Brainvision software and then analyzed using Rhythm, our open source custom MATLAB program (Laughner et al., 2012). The optical action potential signal was filtered with a 100Hz low-pass filter, 3 × 3 binning, and a 1st order drift correction. Activation maps of the isolated atrial tissues were generated based on the maximum derivatives of the optical signals (dVm/dt_{max}). Activation maps of the human tissue paced at a cycle length of 1000 ms were used to calculate the transverse conduction velocity. Action potential duration was calculated by measuring the time elapsed between depolarization and 80% of repolarization.

2.7. Microcontroller-controlled slice culture system

Custom electromechanical components were developed and fabricated to monitor and control the critical culture conditions, including media circulation, temperature adjustment, medium oxygenation, electrical stimulation, optical stimulation, and ECG recording. A microcontroller (Teensy 3.2, PJRC, Sherwood, OR) was used to monitor and actively control each component of the system. The microcontroller interfaced with the rest of the system via a custom breakout board. To achieve medium circulation, we prototyped a peristaltic pump using 3D printing techniques to achieve the appropriate flow rate with low power consumption. To maintain medium temperature and oxygenation, we fabricated a gas-exchanger with built in thermofoil heater (Minco, Minneapolis, MN) using a 3-axis CNC mill (Roland DGA, Irvine, CA). Since a 5.5 V battery was used to power the entire system, a voltage boost regulator (LMR62010, Texas Instrument, Dallas, TX) was used to drive the heater at 12V. A gas permeable PDMS sheet separated the oxygen from the culture medium in the gas exchanger. A humidity and temperature sensor (HTU21D, SparkFun, Boulder, CO) was mounted onto the thermofoil heater to prevent overheating of the culture medium and to detect system leakage.

Each tissue chamber is instrumented with an array of sensors and actuators to monitor the culture condition and to stimulate the tissue to minimize dedifferentiation. To monitor the temperature inside the culture chambers, a high-precision platinum temperature sensor (Digikey, Thief River Falls, MN) was embedded into each chamber. The temperature signal was digitized by a high-precision analog-to-digital converter (ADS1220, Texas Instrument, Dallas, TX) and recorded by the microcontroller. Using the culture chamber temperature, a negative feedback control loop was used to control the heaters to actively maintain a stable culture medium temperature. To record far-field pseudo ECG of cultured tissue, silver/silver chloride sensing electrodes were fabricated into the culture chambers. The pseudo ECG was amplified 1000 times with an operational amplifier, and digitized via a multichannel high sampling rate analog-to-digital converter (ADS131A04, Texas Instruments, Dallas, TX). The ECG signal was recorded by the microcontroller at a 2 kHz sampling rate for further processing. Electrical stimulation of the slices was achieved with field stimulation via platinum/iridium electrodes. For optical stimulation of cultured optogenetic tissue, a 470 ± 10 nm LED (Wurth Electronics, Niedernhall, Germany) was built into each well. All mechanical components were designed in AutoCAD and fabricated with a 3D printer, a laser cutter, and a 3-axis CNC mill.

2.8. ECG data analysis

A custom MATLAB program (Fig. 6) was used to analyze the pseudo ECG recorded from the culture chambers in the heart-on-a-chip system. First, the signals were filtered with a 60Hz notch filter to remove the 60-cycle noise. The signal was then filtered with a 5th order band-pass Butterworth filter with a lower cutoff frequency at 5Hz and a higher cutoff frequency at 100Hz before peak detection was performed on the signal for heart rate calculations.

2.9. Peristaltic pump fabrication

The 3D-printed components of the peristaltic pump were printed on a Stratasys Fortus 250mc in acrylonitrile butadiene styrene plus (ABSP) plastic at a layer height of 0.254 mm in solid fill. In order to minimize the friction caused by moving parts, we implemented frictionless bearings in the pump. The peristaltic pump consists of a single 3D-printed rotor containing 6 rollers, each made up of 7 individual frictionless bearings to reduce the friction caused by the compression of the tubing during rotor rotation. A rod that is centered at the top of the roller stabilizes the rotational axis of the rotor. The underside of the rotor features an aperture that connects to an off-the-shelf 6V DC stepper motor with built-in reduction gears with 30 rpm rotational speed. The motor is held in place by the legs of the 3D-printed rotor housing as shown in Fig. 4B. To facilitate frictionless rotation, one frictionless bearing is secured into the floor of the housing at the interface of the rotor and the motor, while another rests at the interface of the rotor and lid. The housing features an adjustable wall that can be adjusted to accommodate different sized tubing. The pump is controlled and powered by an Arduino motor shield (1438, Adafruit, New York City, NY) with pulse width modulation.

2.10. Statistical analysis

All data presented here are shown as mean ± standard deviation. The statistical analysis was performed using one-way ANOVA followed by a Dunnett's multiple comparisons test.

3. Results

With preserved extracellular matrix and native cell-cell coupling, vibratome-cut human cardiac slices have been demonstrated as an authentic model of the adult human heart for physiological studies and pharmacological testing (Brandenburger et al., 2012; Camelliti et al., 2011; Kang et al., 2016). We have previously established a protocol for obtaining viable human cardiac slices from non-failing donor hearts that were rejected for

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

transplantation and from end-stage failing hearts (Kang et al., 2016). In this project, we present an improved conventional culture method to extend viability length of the cardiac slices *in vitro*. Using optical mapping, we evaluated the conduction parameters of the culture cardiac slices. To achieve greater control over individual aspects of the culture environment, we developed an automated heart-on-a-chip system that supports media circulation, oxygenation, temperature control, electrical stimulation, and static mechanical loading. The system provides a user-friendly platform for optimizing the slice culture condition to achieve long-term culture while maintaining adult cardiomyocyte phenotype. The system is also entirely self-contained to allow for shipping of live human cardiac tissue.

3.1. Prolonged culture of human cardiac slices

Extending the culture length of the cardiac slices while preserving the mature phenotype would enable the study of human cardiac physiology and investigation of chronic pharmacological perturbation and gene therapies. Without microvasculature perfusion, the cardiac slices rely entirely on passive diffusion of oxygen and nutrients. At 380 μm, the thickness of the slices approaches the diffusion limit of oxygen in soft tissue (Barclay, 2005). We previously cultured the cardiac slices with a liquid-air interface that facilitated oxygenation of the slices and was able to preserve normal electrophysiology for 24 h (Kang et al., 2016). In this project, we modified the culture protocol to increase oxygenation of the liquid culture medium. A tri-gas incubator with 30% O_2, 5% CO_2 at 37 °C was used to culture the slices. The slices were individually cultured in 6 well plates with 3 mL of medium in each well. The culture plates were placed on an orbital shaker set at 20 rpm to further increase the dissolved oxygen concentration in the liquid culture medium.

Human cardiac slices obtained from the left ventricular free wall remained electrically viable for up to 21 days *in vitro* and routinely maintained normal electrophysiology for up to 4 days, as shown in Fig. 1. Slices were obtained from multiple hearts to account for inter-heart variability and at a similar transmural depth to control for regional differences in electrophysiology (Kang et al., 2017). We performed optical mapping to measure the conduction parameters of the cardiac slices. The CV was measured at 1 Hz pacing. When compared with fresh slices, the cultured slices maintained anisotropic conduction (Fig. 1D and E), and uniform repolarization across the entire slice (Fig. 1F and G). The slices demonstrated preserved physiological transverse CV for 4 days in culture (Day 0: 21.3 ± 4.5 cm/s, Day 2: 19.7 ± 1.8 cm/s, Day 4: 17.2 ± 1.7 cm/s, Day 0 vs. Day 2: $p = 0.76$, Day 0 vs. Day 4: $p = 0.14$) as shown in Fig. 1A. No significant change in APD was observed in slices cultured for 4 days (Day 0: 372 ± 31 ms, Day 2: 387 ± 16 ms, Day 4: 398 ± 8.6 ms, Day 0 vs. Day 2: $p = 0.68$, Day 0 vs. Day 4: $p = 0.25$) as shown in Fig. 1B. However, significant reduction in the transverse CV was observed in the slices cultured for 21 days (6.7 cm/s). The slowed conduction in the 21-day slice culture may be a manifestation of tissue remodeling and dedifferentiation due to the lack of electrical and mechanical loading. By culturing human cardiac tissue slices at 30% oxygen on an orbital shaker, we were able to significantly prolong the culture duration while maintaining normal electrophysiology. With this in mind, we developed a gas exchanger for our heart-on-a-chip system to achieve precise control of medium oxygenation over a greater range.

3.2. Heart-on-a-chip system

To prolong the culture period of human cardiac slices and prevent tissue dedifferentiation, we developed a heart-on-a-chip system for organotypic culture of human cardiac slices *in vitro* with custom electronic and microfluidic components, as shown in Fig. 2. The culture system continuously monitors and maintains stable culture conditions, including culture medium temperature, circulation, and oxygenation. The culture chambers include actuators and sensors for electrical stimulation, sensing, and optical stimulation. Unlike cell lines that could be cryopreserved for shipping, human cardiac slices are susceptible to hypoxia when not maintained properly, thus complicating shipping of live slices across long distances. This culture system that we have developed is fully self-contained with an integrated power supply, oxygen source, and media reservoir, allowing the maintenance of tissue viability during transportation. To increase culture capacity and reduce the cost of repair, we designed the culture system to be modular for plug and play operation.

The culture conditions inside the heart-on-a-chip system are closely monitored and adjusted by an array of sensors and actuators controlled by a microcontroller, as shown in Fig. 2A. The system consists of a custom control module, multiple culture chambers, pumps, heaters, a gas exchanger, a culture medium reservoir, a gas pressure regulator, and a gas tank filled with pure oxygen. With an average current draw of below 600 mA, the culture system has an operation time of three days on a single portable 40,000 mAh battery, which is sufficient for overnight shipping. All culture parameters can be independently adjusted in real time to optimize and maintain the culture condition for human cardiac slices.

3.3. Smart tissue culture chamber

Electrical stimulation and static mechanical stimulation has been shown to maintain the structural and functional properties of isolated adult rat ventricular myocytes (Berger et al., 1994; Folliguet et al., 2001; Simpson et al., 1996). To minimize tissue dedifferentiation and to monitor the culture parameters, we instrumented each culture chamber with an array of actuators and sensors, including field-pacing electrodes, sensing electrodes, a temperature sensor, and a light source for optogenetic stimulation and fluorescence recordings (Fig. 3B). The bottom of each chamber is coated with polydimethylsiloxane (PDMS) to allow for the mechanical anchoring of the slices using miniature dissection pins in order to provide a static mechanical stretch. To avoid physical damage to the tissue from point pacing electrodes, platinum-iridium (Pt/Ir) electrodes, which have proven biocompatibility and low electrical resistance, were fabricated into the culture chamber for field stimulation. The default stimulation parameters were set at 3V pulse amplitude, 5 ms pulse duration, and 1 s pacing cycle length. As shown in Fig. 3D, no undesirable voltage fluctuations were observed in the recorded stimulation waveform. The pacing parameters are user adjustable to allow for optimization of the pacing protocol during culture in case of changes in pacing capture threshold or specific protocol requirements.

Temperature of the culture medium was maintained by a feedback control system based on the temperature inside the tissue culture chamber via a platinum resistance thermometer. Based on platinum's linear resistance-temperature relationship, an analog temperature signal was obtained by comparing the voltage from the platinum thermometer to a reference voltage with an instrumentation amplifier. The analog signal was then digitized with an analog-to-digital converter before being recorded and converted to Celsius by the microcontroller at a sampling rate of 1Hz. We developed two configurations of the culture chambers, as shown in Fig. 3A,C. While both configurations perform similarly, the design shown in Fig. 3A is much more compact, with all electronic components integrated onto the same printed circuit board, whereas the system in Fig. 3C utilizes a modular design for the ease of

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

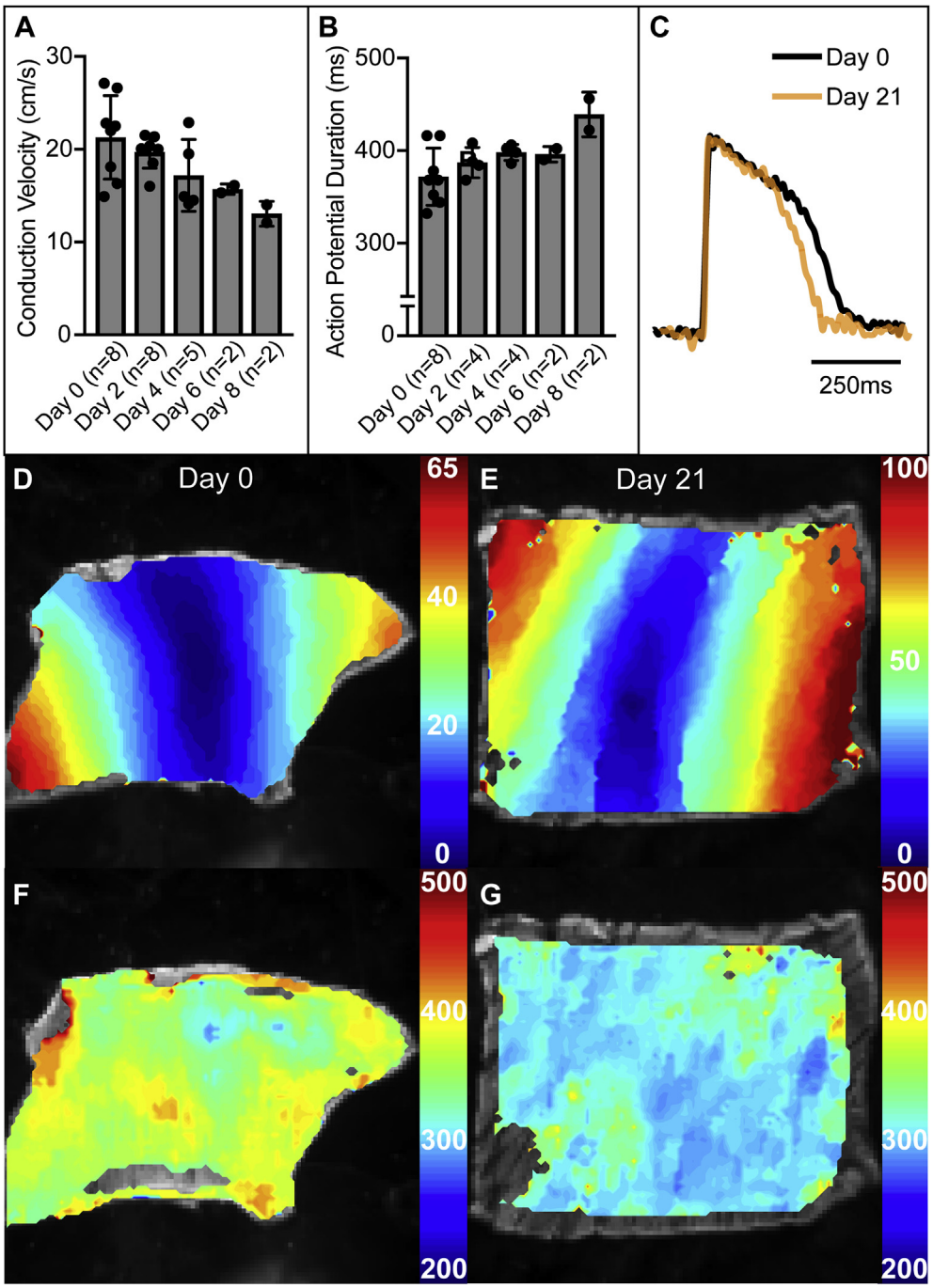

Fig. 1. Cultured human cardiac slice electrophysiology. A) Transverse conduction velocity of cultured human cardiac slices over time. B) Action potential duration of cultured human cardiac slices. No significant changes in CV and APD were observed in slices cultured for 2 days and 4 days when compared with fresh slices. C) Comparison of action potentials of a fresh human cardiac slice and a slice cultured for 21 days. D) Activation map of the fresh slice. Colorbars represent activation times in ms. E) Activation map of the cardiac slice cultured for 21 days. F) APD map of the fresh slice shown in panel D. Colorbars represent APD in ms. G) APD map of the 21-day slice shown in panel E.

scaling up culture capacity with plug and play operation.

Due to the scarcity of human heart tissue, isolated murine atrial preparations containing the sinus node were used to test and optimize the culture system during development. Viability of the culture murine atrial preparation was measured by its intrinsic heart rate. To monitor the murine atrial sinus rhythm in culture, we fabricated Pt/Ir sensing electrodes into the culture chambers for pseudo ECG recording. The pseudo ECG was amplified 1,000x with an operational amplifier and quantized via a multichannel analog-to-digital converter at a 2 kHz sampling rate. The ECG signal was recorded by the microcontroller for post processing.

3.4. Custom gas exchanger

Since the thickness of human cardiac slices approaches the limit of oxygen diffusion, sufficient oxygenation of the culture medium is critical to maintaining tissue viability (Barclay, 2005). When cultured in a regular incubator with 20% O_2, the core of the tissue slice may experience hypoxia, causing altered gene expression and reducing tissue viability (Giordano, 2005; Huang, 2004). By

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

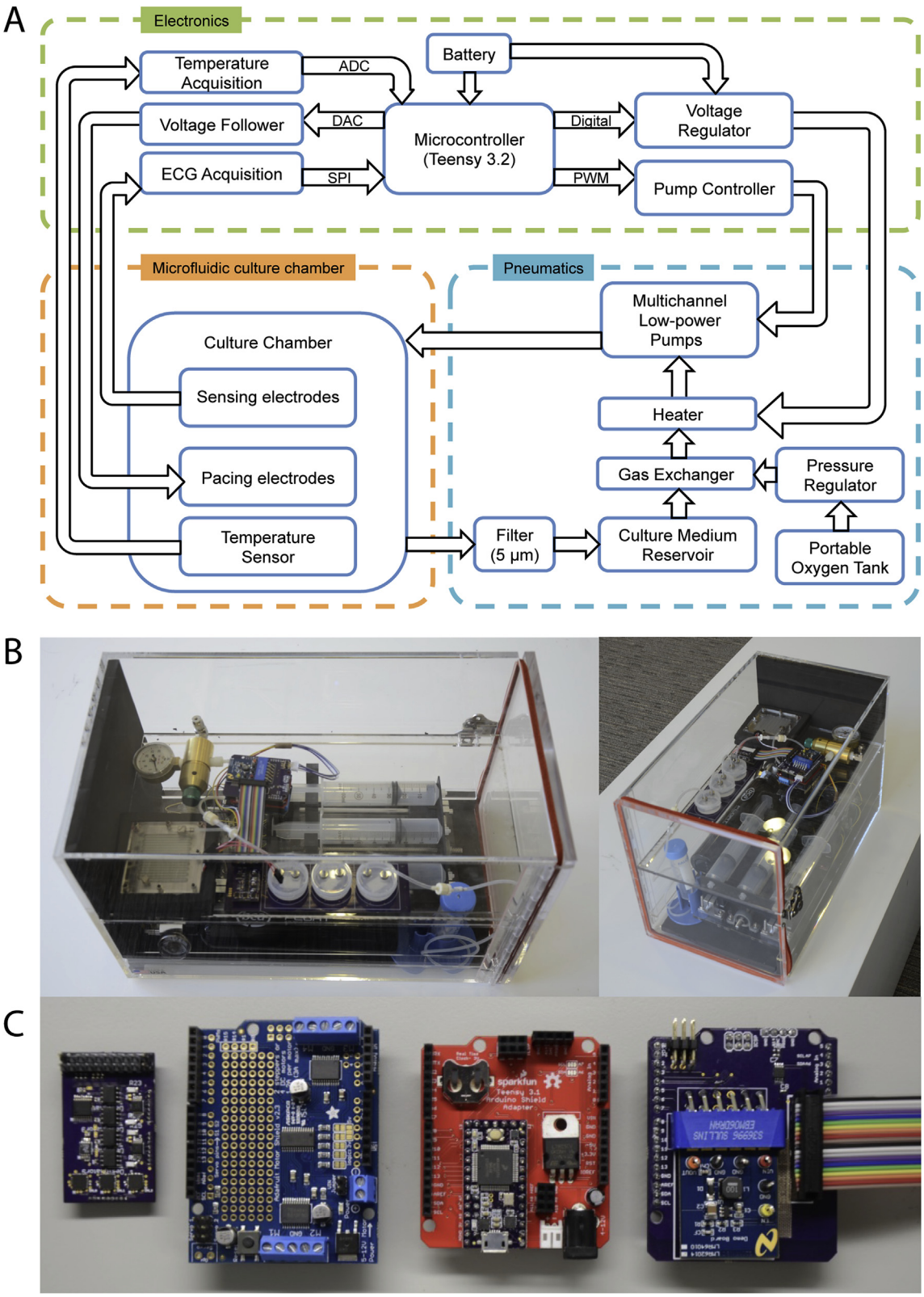

Fig. 2. Human heart-on-a-chip system. A) Block diagram of the culture system. The system consists of custom electronics and electromechanical components to maintain stable culture conditions. B) Pictures of the assembled system. Insulation foam was removed from the sides of the enclosure for illustration. C) Modular electronic control unit. The electronic components consist of a signal acquisition module, a motor driver, a microcontroller, and a power module (from left to right).

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

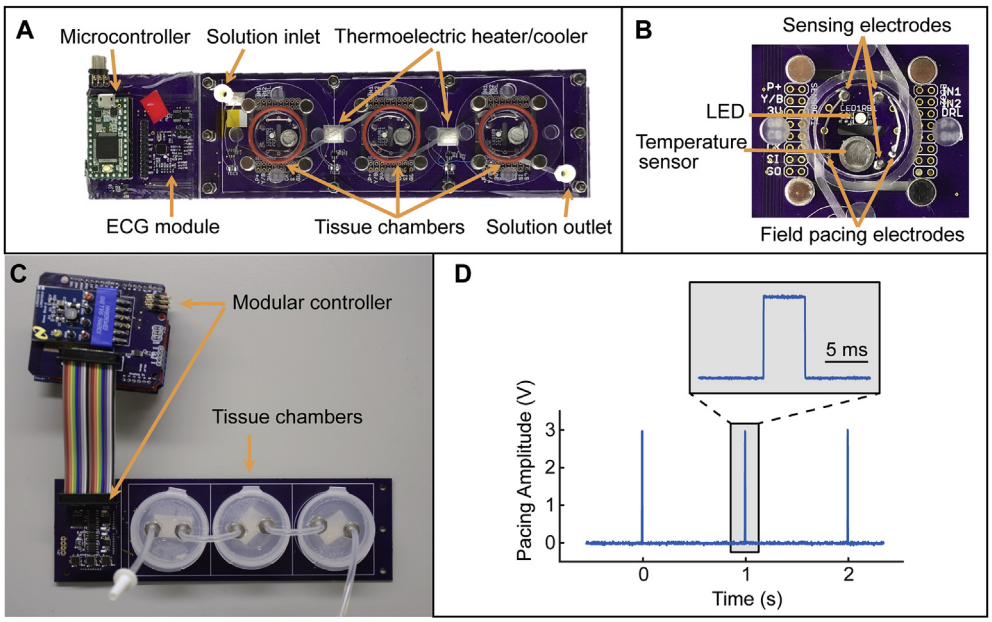

Fig. 3. Smart tissue culture chamber overview. A) A fully integrated culture device for compactness. B) Culture chambers intrumented with sensing electrodes, field pacing electrodes, a temperature sensor, and a LED. C) Modular culture system for ease of scaling up culture capacity. D) Recorded waveform during electrical field stimulation with 3 V pulse amplitude, 5 ms pulse duration, and 1 s pacing cycle length.

culturing human cardiac slices at 30% oxygen on an orbital shaker, we were able to prolong the culture duration while maintaining normal electrophysiology. To supply sufficient oxygen to the slices cultured in the smart tissue chamber, a custom gas exchanger was developed to oxygenate the culture medium before circulation to the culture chambers, as shown in Fig. 4A. The gas exchanger consists of two mirrored chambers separated by a 0.35 mm thick gas-permeable PDMS membrane. One chamber of the gas

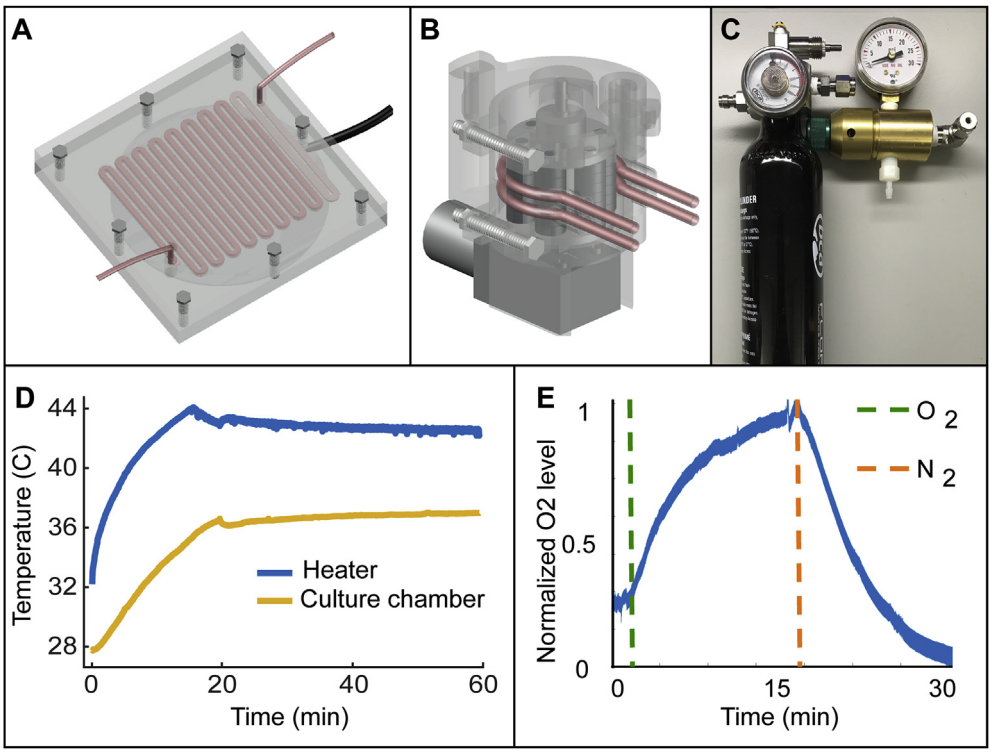

Fig. 4. Custom gas exchanger, heater, and pump for maintaining culture medium oxygenation, temperature, and circulation. A) Rendering of the CNC milled gas exchanger. The top chamber is made of polycarbonate for liquid medium to pass through. The bottom chamber is made of stainless steel and is heated with a thermofoil heater. B) Rendering of the 3D printed peristaltic pump. C) Portable gas tank and miniature pressure regulator. D) Recorded temperatures of the heater and the culture chamber. The culture chamber temperature rapidly reached and maintained physiological temperature with miminal fluctuation. E) Dissolved oxygen level in culture medium with different gas. The oxygen concentration in the liquid medium rapidly reached saturation when the gas exchanger was filled with oxygen. The dissolved oxygen was depleted when the gas exchanger was filled with nitrogen.

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

exchanger is pressurized with pure oxygen at 15psi, while the culture medium flows through the mirrored chamber. The total surface area for oxygen exchange is 17 cm^2. Using an oxygen sensor (ADInstruments, Colorado Springs, CO), we measured a dissolved oxygen concentration of 1.3 mM in the culture medium, 5–6 times higher than that of conventional culture in a cell incubator (McMurtrey, 2016). As shown in Fig. 4E, gas exchange can rapidly occur inside the culture system. When the gas exchanger was filled with pure oxygen, the diffused oxygen level reached saturation in approximately 15 min. Vice versa, when the oxygen was purged and nitrogen was fed into the gas exchanger, the oxygen concentration in the culture medium was depleted in approximately 15 min. To maintain tissue viability during transportation, the culture system also contains a portable small gas tank and a miniature pressure regulator (Fig. 4C). The gas tank can be pressurized to 2100 psi, allowing for an estimated 3 weeks of oxygen supply.

3.5. Maintenance of stable culture temperature

Since most enzymes denature rapidly at high temperatures and ion channel conductance is temperature dependent, the ability to maintain stable temperature inside the culture chambers is critical for preserving the viability and normal electrophysiology of cardiac slices (Dumaine et al., 1999; Milburn et al., 1995; Voets et al., 2004). We implemented a proportional control, a type of feedback control system, to maintain a stable culture medium temperature and to compensate for the changing ambient temperature. Two thermofoil heaters were built into the gas exchanger and the medium reservoir, where the liquid medium has the greatest surface-to-volume ratio. Since heat transfer to the culture medium does not stop immediately when the heaters are powered off due to the large heat capacitance of the heaters, a proportional control system was used to avoid undesirable temperature fluctuations in the culture chambers, as shown in the following equation. To prevent overheating of the culture medium, the temperature of the heaters was monitored and limited to a maximum of 45 °C, well below the denaturation temperature of fetal bovine serum. As shown in Fig. 4D, the culture medium inside the culture chamber reached 37 °C from room temperature within 20 min with minimal overshoot. The temperature was subsequently maintained without significant fluctuations.

$$P_{out} = K_p \times e(t) + p0$$

Equation for proportional control, where p0 is output with zero error and is set at 37 °C, e(t) is the instantaneous error at time t and is the difference between p0 and culture chamber temperature, K_p is a proportional gain and is set at 1, P_{out} is the target temperature of the heater. The upper bound of P_{out} is set to 45 °C to avoid overheating of the culture medium.

3.6. Low-power pumps for medium circulation

Both heating and oxygenation of the culture media require circulation of the perfusion medium. A robust means of driving steady flow is critical for maintaining stable heat and gas exchange. We evaluated several pumps for their long-term dependability and low power consumption. We adopted a piezoelectric pump and a custom peristaltic pump for two versions the culture system for use under different cases. With a low power consumption of 250 mW, the piezoelectric pump would be preferred when power is limited, such as during transportation of the culture system. However, since the piezoelectric pump works by rapidly deforming and releasing a piezo element when voltage is applied at a high frequency, the pump requires direct contact with the culture medium and can potentially increase the chance of contamination. For the long-term culture of the cardiac slices when the culture system is connected to an external power source, we developed a custom 3D printed peristaltic pump as shown in Fig. 4B. Since the liquid is forced through a tube when compressed by rollers in a peristaltic pump, the tubing can be sterilized by ethylene oxide or autoclave to minimize the chance of contamination. With a power consumption of 1W, our custom peristaltic pump is 10–15 times more power efficient than similar commercially available pumps. With built-in reduction gears, our peristaltic pump is also significantly more reliable than other low cost peristaltic pumps that drive the rollers via friction coupling. To avoid excess pressure buildup in the gas exchanger, the piezoelectric pump and the peristaltic pump are controlled by pulse width modulation to achieve a stable 2 mL/min flow rate.

3.7. Tissue viability in the heart-on-a-chip system

To evaluate the effectiveness of our culture system in maintaining viability of cardiac tissue, we performed optical mapping of the cardiac slices cultured in the system and tracked the automaticity of cultured murine atria. To demonstrate the system's flexibility in programmed pacing, the human cardiac slices were paced at 1Hz with 5 ms pulse width for 10 min every hour. As shown in Fig. 5A–C, the human cardiac slices cultured in the heart-on-a-chip system remained electrically viable for up to 3 days. When compared with a freshly sectioned slice (Fig. 5A), the slices cultured for 1 day (Fig. 5B) and 3 days (Fig. 5C) demonstrated preserved anisotropic conduction and normal action potential morphology (Fig. 5D). Greater noise was observed in the optical action potential recorded from the human cardiac slice cultured for 3 days, suggesting declining tissue viability. To achieve longer culture duration, optimization in terms of the medium flow rate, oxygenation, medium composition, and the electrical stimulation protocol is necessary, all of which are easily adjustable with our culture system.

Isolated murine atrial preparation has been used to study atrial conduction and pacemaking (Choate and Feldman, 2003; Glukhov et al., 2010b; Swaminathan et al., 2011). The preparation can be maintained in culture for extended period due to the thickness of the tissue. During development of the culture system, isolated murine atrial preparation was used to test the culture system. Since the sinoatrial node is preserved in the preparation, automaticity of the murine atria can be tracked as a measure of tissue viability. As shown in Fig. 5E, the cultured murine atria exhibited stable physiological heart rate in the culture system (Mitchell et al., 1998). To reduce motion artifacts in the far-field electrical recording, the system was programmed to power down medium circulation and heating during recording. As shown in Fig. 5F, a clean atrial electrical signal could be recorded for heart rate calculation.

We developed custom MATLAB program and a graphic user interface (GUI) to monitor the performance of the culture chamber and the condition of culture murine atria, as shown in Fig. 6. The top panel of the GUI shows the temperatures of the heaters and the culture chambers over time for tracking heater and pump malfunction. The bottom three panels show far-field electrical recordings from the culture chambers. The recorded signal is filtered by a 2nd order Butterworth notch filter to remove the 60Hz power line interference and a 5th order band-pass Butterworth filter with a lower cutoff frequency at 5Hz and a higher cutoff frequency at 100Hz to remove drift and additional noise in the signal. Peak detection with user selectable settings can be performed on the electrical recordings for heart rate calculation.

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

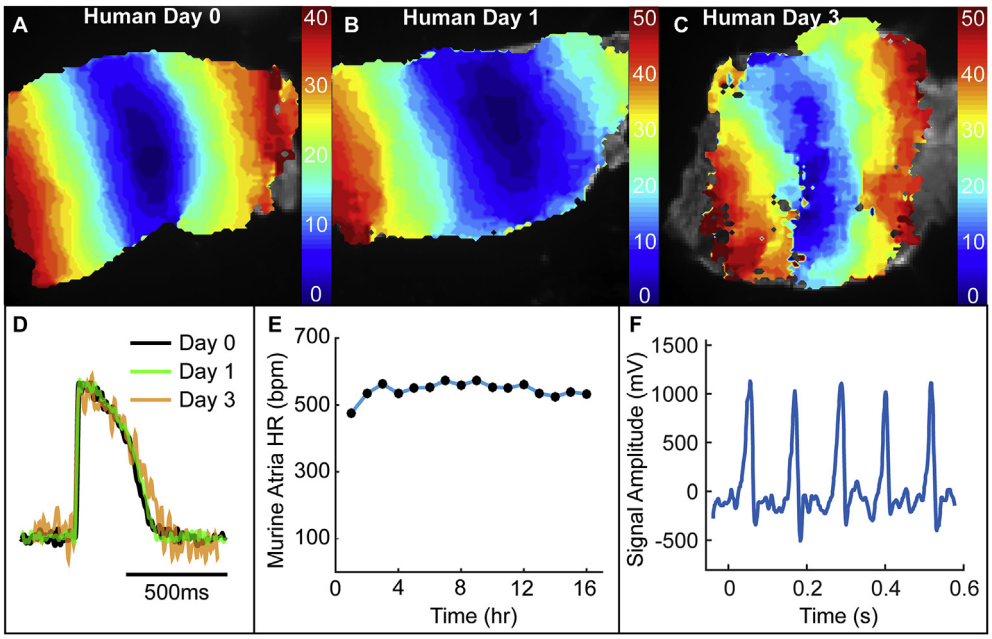

Fig. 5. Organotypic culture of human and murine cardiac tissue in the heart-on-a-chip system. A-C) Activation maps of a fresh human cardiac slice and slices maintained for 1 and 3 days in the culture system. The colorbars represent activation times in ms. D) Action potentials recorded from the slices using optical mapping. E) The heart-on-a-chip system maintained stable heart rate of the cultured murine atrial preparation. F) Far-field recording of cultured murine atrial preparation.

4. Discussion

Previously, we demonstrated the advantages of human cardiac slices as a model for studying human cardiac physiology and for drug efficacy and toxicity testing (Kang et al., 2016). However, the limited culture duration and the intricate culture protocol confined the use of human cardiac slices to acute studies. Here, we present an improved culturing method that maintains normal electrophysiology of the human cardiac slices for 4 days and developed an automated, self-contained heart-on-a-chip system as a user-friendly platform for further optimization of slice culture conditions.

Several studies have demonstrated the feasibility of maintaining cardiac slice viability in culture but have observed significant tissue remodeling in the slices cultured long-term. In the absence of electrical and/or mechanical stimulation, cardiomyocytes undergo significant remodeling and dedifferentiation, evident by diminished contractile force, triangulation of action potential morphology, and reduced gap junction expression (Brandenburger et al., 2012; Kaneko et al., 2012). We demonstrated that the cultured human cardiac slices maintain normal electrophysiology for up to 4 days and remain electrically viable for up to 21 days when cultured inside a high oxygen environment. Significant reduction in CV was observed in the cardiac slices cultured longer than 4 days. The reduction in CV of the cultured slice is likely due to tissue dedifferentiation in the absence of electrical and mechanical stimulation. To overcome the limitations associated with conventional culture methods, we subsequently developed a culture system with electrical stimulation and static mechanical stretch capabilities as a platform for optimizing organotypic culture of the human cardiac slices.

We utilized optical mapping to characterize the conduction parameters of the cardiac slices at a high spatial and temporal resolution. Optical mapping is also capable of measuring other functional parameters such as the intracellular calcium and the metabolic state, using calcium-sensitive fluorescent dyes and NADH fluorescence (Lou et al., 2011b; Moreno et al., 2017). However, the use of fluorescent probes in optical mapping hampers its ability to take repeated measurements on the same slice over the length of culture. To overcome this, other techniques such as intracellular microelectrode recording and multi-electrode array recording can be applied to study the cardiac slices (Camelliti et al., 2011).

The prolonged culture of human cardiac slices demonstrated here enables the study of chronic drug effects, gene therapies, and gene editing. Adenoviral (Ad) vectors are a promising approach for *in vivo* gene delivery because of the ease of producing high titer when compared with lentivirus and the larger packaging capacity when compared with adeno-associated virus (Thomas et al., 2003). However, the clinical adoption of Ad vectors for gene therapy has been limited by its dependence on the coxsackievirus and adenovirus receptor (CAR) for transduction (Dmitriev et al., 1998). With the preserved native extracellular matrix, the cardiac slices are a powerful platform for testing advancements in vector technology, such as tropism-modified CAR-independent Ad5 vectors.

With the prolonged culture length, human cardiac slices can be used as an accurate model of the human myocardium for testing the effect of exogenous gene expression. Optogenetic stimulation and inhibition with light-gated ion channels such as Channelrhodopsin-2 (ChR2) and anion channel rhodopsins (ACRs) has been proposed as a selective and safe method of cardiac pacing and cardioversion (Govorunova et al., 2015; Jia et al., 2011). With built-in LED light source and far field-sensing electrodes, our culture system can perform automated evaluations of optogenetic stimulation on specific regions of the adult human heart. RNA interference (RNAi) has been proposed as a potential therapeutic and research tool. The ability to silence specific genes of interest with small interfering RNA (siRNA) and short hairpin RNA (shRNA) makes RNAi a powerful tool for studying cardiac physiology (Poller et al., 2010; Suckau et al., 2009). The approach has been used for suppressing inflammatory response and oxidative stress to improve cardiac function following myocardial infarction in animal models (Hong et al., 2014; Somasuntharam et al., 2013). When applied to human cardiac slices, RNAi can be used to gain valuable

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

Fig. 6. Custom monitoring and analysis software. The main graphical user interface consists of four sections. Section 1 is for loading the devices log and for selecting ECG recordings. Section 2 shows a history of the device temperature. Section 3 shows the psudo ECG recorded from the cultured chambers. Section 4 is used for performing heart rate calculation with user selectable peak detection parameters.

insights to human cardiac physiology by selective knockdown of specific ion channels and subunits.

To overcome the limitations associated with conventional culture methods and to achieve precise control over individual culture conditions, we developed a heart-on-a-chip system in which different culture parameters can be individually adjusted to establish the optimal culture condition. We also designed our system to be entirely self-contained to support transport of live cardiac slices. Using preset parameters and a feedback control system, the culture system maintains stable temperature, circulation, and oxygenation of the culture medium. The culture chambers are instrumented with an array of actuators and sensors for electrical stimulation, mechanical anchoring for static stimulation, electrical recording, and optogenetic stimulation and sensing.

Continual electrical stimulation of isolated adult rat cardiomyocytes was found to preserve contractility, evident by the preserved amplitude of contraction, the velocities of shortening and relaxation, and the peak calcium current density (Berger et al., 1994). In the field of tissue engineering, electrical stimulation was also shown to improve expression of major cardiac markers and induced cell alignment and coupling in hiPSC-CMs (Radisic et al., 2004). With built-in field pacing electrodes, our culture system allows for testing of electrical stimulation protocols with different frequencies and durations to establish the optimal protocol for minimizing tissue dedifferentiation. Pt/Ir was chosen as the material for the pacing electrodes to avoid release of free radicals that could cause oxidative stress to the tissue. Any proton gradient generated by the electrical field would be dissipated by the

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001

circulation of the culture medium. In extreme cases where continuous high frequency pacing might be required to maintain tissue phenotype, electrolysis of the culture medium would break down sodium chloride and water molecules to form sodium hydroxide, causing an increase in pH of the culture medium. A collaborative effort to develop a miniature pH sensor with minimal baseline drift is underway. The addition of a pH sensor will allow for real-time adjustment of the culture medium pH in near future.

To maintain a stable temperature in the culture system, we evaluated the effectiveness of three types of feedback control systems, including on-off control, proportional control, and proportional-integral-derivative (PID) control. Also known as a hysteresis controller, an on-off controller rapidly switches the power state of the heaters based on the temperature inside the culture chambers and is the easiest to implement. However, the on-off controller does not compensate for the delayed heat exchange between the heaters and the culture medium, causing large temperature oscillations. On the other hand, a PID controller can achieve stable temperature control for a given system configuration when the proportion, integral, and derivative terms are well characterized. However, the stringentness of a PID controller hinders its ability to adjust to changing system configurations. Therefore, the proportional controller was implemented in our culture system to achieve a stable temperature while allowing for plug and play operation of the culture chambers when expanding the culture capacity.

For future work, we aim to further optimize the organotypic culture of human cardiac slices by systematically testing individual culture parameters of the heart-on-a-chip system and to develop technologies for multiparametric functional characterization of cultured cardiac slices to achieve automated testing of drugs, gene therapies, and gene editing, as illustrated in Fig. 7. Building on our current heart-on-a-chip system, we will develop a microelectrode array system for real-time monitoring of CV and APD and a compact optical detection system for measuring transmembrane potential, intracellular calcium dynamics, and metabolic function. To further optimize the culture condition, we will develop technologies for real-time adjustment of culture medium pH by modifying the ratio of pure oxygen and O_2/CO_2 mixture in the gas exchanger based on continuous readings from an integrated pH sensor. The development of an automated heart-on-a-chip platform with organotypic human cardiac slices would accelerate pre-clinical drug testing and research in human cardiac physiology. Integration of heart-on-a-chip with other human organ tissue slices and/or human iPSC-derived cells/tissues will support human-on-a-chip systems for physiology investigations.

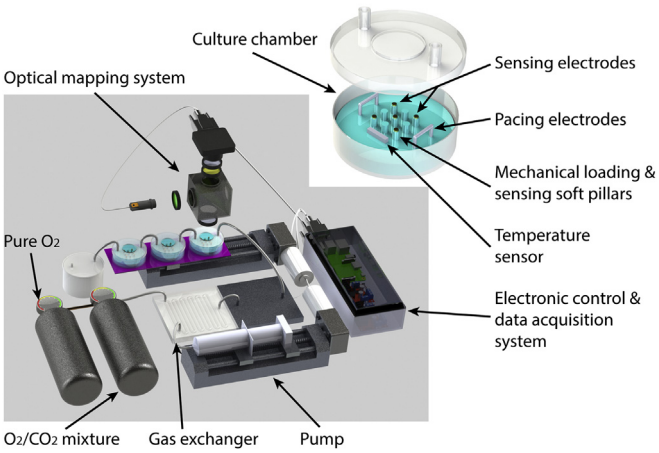

Fig. 7. Future work on automated multiparametric characterization of cardiac slices. Miniaturized optical mapping system will be used for measuring action potential, calcium transient, and metabolic state of cultured slices on a motorized stage. Maintenance of the culture medium pH will be achieved by controlling the ratio of pure oxygen and O_2/CO_2 mixture in the gas exchanger. A multi-electrode array system will be implemented in the culture chambers for real-time functional monitoring of the slices.

Acknowledgements

We are grateful to the Washington Regional Transplant Community and families of the donors. We also thank all members of the Efimov lab and the Kay Lab at George Washington University, especially Dr. Chaoyi Kang, Jaclyn Brennan, Dr. Sharon George, Dr. Kedar Aras, Dr. Rokhaya Faye, and Frederick Zasadny for the critical discussion of the project. We are grateful to Dr. Stacey Rentschler and Dr. Nathaniel Huebsch for their expert technical advice. This project was funded by National Institutes of Health (grants R01 HL114395 and R01 HL126802) and the Leducq Foundation (grant RHYTHM).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pbiomolbio.2018.06.001.

References

Barclay, C.J., 2005. Modelling diffusive O2 supply to isolated preparations of mammalian skeletal and cardiac muscle. J. Muscle Res. Cell Motil. 26, 225–235. https://doi.org/10.1007/s10974-005-9013-x.

Berger, H.J., Prasad, S.K., Davidoff, A.J., Pimental, D., Ellingsen, O., Marsh, J.D., Smith, T.W., Kelly, R.A., 1994. Continual electric field stimulation preserves contractile function of adult ventricular myocytes in primary culture. Am. J. Physiol. 266, H341–H349. https://doi.org/10.1152/ajpheart.1994.266.1.H341.

Bird, S.D., Doevendans, P.A., Van Rooijen, M.A., Brutel De La Riviere, A., Hassink, R.J., Passier, R., Mummery, C.L., 2003. The human adult cardiomyocyte phenotype. Cardiovasc. Res. 58, 423–434. https://doi.org/10.1016/S0008-6363(03)00253-0.

Brandenburger, M., Wenzel, J., Bogdan, R., Richardt, D., Nguemo, F., Reppel, M., Hescheler, J., Terlau, H., Dendorfer, A., 2012. Organotypic slice culture from human adult ventricular myocardium. Cardiovasc. Res. 93, 50–59. https://doi.org/10.1093/cvr/cvr259.

Bussek, A., Schmidt, M., Bauriedl, J., Ravens, U., Wettwer, E., Lohmann, H., 2012. Cardiac tissue slices with prolonged survival for in vitro drug safety screening. J. Pharmacol. Toxicol. Meth. 66, 145–151. https://doi.org/10.1016/j.vascn.2011.12.002.

Camelliti, P., Al-Saud, S.A., Smolenski, R.T., Al-Ayoubi, S., Bussek, A., Wettwer, E., Banner, N.R., Bowles, C.T., Yacoub, M.H., Terracciano, C.M., 2011. Adult human heart slices are a multicellular system suitable for electrophysiological and pharmacological studies. J. Mol. Cell. Cardiol. 51, 390–398. https://doi.org/10.1016/j.yjmcc.2011.06.018.

Chi, K.R., 2013. Revolution dawning in cardiotoxicity testing. Nat. Rev. Drug Discov. 12, 565–567. https://doi.org/10.1038/nrd4083.

Choate, J.K., Feldman, R., 2003. Neuronal control of heart rate in isolated mouse atria. Am. J. Physiol. Heart Circ. Physiol. 285, H1340–H1346. https://doi.org/10.1152/ajpheart.01119.2002.

Coppini, R., Ferrantini, C., Aiazzi, A., Mazzoni, L., Sartiani, L., Mugelli, A., Poggesi, C., Cerbai, E., 2014. Isolation and functional characterization of human ventricular cardiomyocytes from fresh surgical samples. J. Vis. Exp. 1–14. https://doi.org/10.3791/51116.

Dmitriev, I., Krasnykh, V., Miller, C.R., Wang, M., Kashentseva, E., Mikheeva, G., Belousova, N., Curiel, D.T., 1998. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. J. Virol. 72, 9706–9713.

Dumaine, R., Towbin, J., Brugada, P., Vatta, M., Nesterenko, D.V., Nesterenko, V.V., Brugada, J., Brugada, R., Antzelevitch, C., 1999. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. Circ. Res. 85, 803–809. https://doi.org/10.1161/01.RES.85.9.803.

Esch, M.B., Ueno, H., Applegate, D.R., Shuler, M.L., 2016. Modular, pumpless body-on-a-chip platform for the co-culture of GI tract epithelium and 3D primary liver tissue. Lab Chip 16, 2719–2729. https://doi.org/10.1039/C6LC00461J.

Folliguet, T.A., Rücker-Martin, C., Pavoine, C., Deroubaix, E., Henaff, M., Mercadier, J.J., Hatem, S.N., 2001. Adult cardiac myocytes survive and remain excitable during long-term culture on synthetic supports. J. Thorac. Cardiovasc. Surg. 121, 510–519. https://doi.org/10.1067/mtc.2001.112528.

Giordano, F.J., 2005. Oxygen, oxidative stress, hypoxia, and heart failure. J. Clin.

Invest. https://doi.org/10.1172/JCI200524408.
Glukhov, A.V., Fedorov, V.V., Kalish, P.W., Ravikumar, V.K., Lou, Q., Janks, D., Schuessler, R.B., Moazami, N., Efimov, I.R., 2012. Conduction remodeling in human end-stage nonischemic left ventricular cardiomyopathy. Circulation 125, 1835–1847. https://doi.org/10.1161/CIRCULATIONAHA.111.047274.
Glukhov, A.V., Fedorov, V.V., Lou, Q., Ravikumar, V.K., Kalish, P.W., Schuessler, R.B., Moazami, N., Efimov, I.R., 2010a. Transmural dispersion of repolarization in failing and nonfailing human ventricle. Circ. Res. 106, 981–991. https://doi.org/10.1161/CIRCRESAHA.109.204891.
Glukhov, A.V., Flagg, T.P., Fedorov, V.V., Efimov, I.R., Nichols, C.G., 2010b. Differential KATP channel pharmacology in intact mouse heart. J. Mol. Cell. Cardiol. 48, 152–160. https://doi.org/10.1016/j.yjmcc.2009.08.026.
Govorunova, E.G., Sineshchekov, O.A., Janz, R., Liu, X., Spudich, J.L., 2015. Natural light-gated anion channels: a family of microbial rhodopsins for advanced optogenetics. Science (80-.) 349, 647–650. https://doi.org/10.1126/science.aaa7484.
Hasenfuss, G., 1998. Animal models of human cardiovascular disease, heart failure and hypertrophy. Cardiovasc. Res. 39, 60–76. https://doi.org/10.1016/S0008-6363(98)00110-2.
Hong, J., Ku, S.H., Lee, M.S., Jeong, J.H., Mok, H., Choi, D., Kim, S.H., 2014. Cardiac RNAi therapy using RAGE siRNA/deoxycholic acid-modified polyethylenimine complexes for myocardial infarction. Biomaterials 35, 7562–7573. https://doi.org/10.1016/j.biomaterials.2014.05.025.
Huang, Y., 2004. Cardiac myocyte-specific HIF-1 deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart. FASEB. J. https://doi.org/10.1096/fj.04-1510fje.
Itzhaki, I., Maizels, L., Huber, I., Zwi-Dantsis, L., Caspi, O., Winterstern, A., Feldman, O., Gepstein, A., Arbel, G., Hammerman, H., Boulos, M., Gepstein, L., 2011. Modelling the long QT syndrome with induced pluripotent stem cells. Nature 471, 225–230. https://doi.org/10.1038/nature09747.
Jia, Z., Valiunas, V., Lu, Z., Bien, H., Liu, H., Wang, H.Z., Rosati, B., Brink, P.R., Cohen, I.S., Entcheva, E., 2011. Stimulating cardiac muscle by light cardiac optogenetics by cell delivery. Circ. Arrhythmia Electrophysiol. 4, 753–760. https://doi.org/10.1161/CIRCEP.111.964247.
Kaneko, M., Coppen, S.R., Fukushima, S., Yacoub, M.H., Suzuki, K., 2012. Histological validation of heart slices as a model in cardiac research. J. Cell Sci. Ther. 3. https://doi.org/10.4172/2157-7013.1000126.
Kang, C., Badiceanu, A., Brennan, J.A., Gloschat, C., Qiao, Y., Trayanova, N.A., Efimov, I.R., 2017. β-adrenergic stimulation augments transmural dispersion of repolarization via modulation of delayed rectifier currents I Ks and I Kr in the human ventricle. Sci. Rep. 7 (1), 15922.
Kang, C., Qiao, Y., Li, G., Baechle, K., Camelliti, P., Rentschler, S., Efimov, I.R., 2016. Human organotypic cultured cardiac slices: New platform for high throughput preclinical human trials. Sci. Rep. 6. https://doi.org/10.1038/srep28798.
Karakikes, I., Ameen, M., Termglinchan, V., Wu, J.C., 2015. Human induced pluripotent stem cell-derived cardiomyocytes: insights into molecular, cellular, and functional phenotypes. Circ. Res. https://doi.org/10.1161/CIRCRESAHA.117.305365.
Laughner, J.I., Ng, F.S., Sulkin, M.S., Arthur, R.M., Efimov, I.R., 2012. Processing and analysis of cardiac optical mapping data obtained with potentiometric dyes. Am. J. Physiol. Heart Circ. Physiol. 303, H753–H765. https://doi.org/10.1152/ajpheart.00404.2012.
Loskill, P., Sezhian, T., Tharp, K.M., Lee-Montiel, F.T., Jeeawoody, S., Reese, W.M., Zushin, P.-J.H., Stahl, A., Healy, K.E., 2017. WAT-on-a-chip: a physiologically relevant microfluidic system incorporating white adipose tissue. Lab Chip 17, 1645–1654. https://doi.org/10.1039/C6LC01590E.
Lou, Q., Fedorov, V.V., Glukhov, A.V., Moazami, N., Fast, V.G., Efimov, I.R., 2011a. Transmural heterogeneity and remodeling of ventricular excitation- contraction coupling in human heart failure. Circulation 123, 1881–1890. https://doi.org/10.1161/CIRCULATIONAHA.110.989707.
Lou, Q., Li, W., Efimov, I.R., 2011b. Multiparametric optical mapping of the Langendorff-perfused rabbit heart. J. Vis. Exp. https://doi.org/10.3791/3160.
Mak, I.W.Y., Evaniew, N., Ghert, M., 2014. Lost in translation: animal models and clinical trials in cancer treatment. Am. J. Transl. Res. https://doi.org/1943-8141/AJTR1312010.
McMurtrey, R.J., 2016. Analytic models of oxygen and nutrient diffusion, metabolism dynamics, and architecture optimization in three-dimensional tissue constructs with applications and insights in cerebral organoids. Tissue Eng. C Meth. 22, 221–249. https://doi.org/10.1089/ten.tec.2015.0375.
Milburn, T., Saint, D.A., Chung, S.H., 1995. The temperature dependence of conductance of the sodium channel: implications for mechanisms of ion permeation. Recept. Channel 3, 201–211.
Mitchell, G.F., Jeron, A., Koren, G., 1998. Measurement of heart rate and QT interval in the conscious mouse. Am. J. Physiol. Heart Circ. Physiol. 274 (3), H747–H751.
Moreno, A., Kuzmiak-Glancy, S., Jaimes, R., Kay, M.W., 2017. Enzyme-dependent fluorescence recovery of NADH after photobleaching to assess dehydrogenase activity of isolated perfused hearts. Sci. Rep. 7. https://doi.org/10.1038/srep45744.
Nerbonne, J.M., Nichols, C.G., Schwarz, T.L., Escande, D., 2001. Genetic manipulation of cardiac K(+) channel function in mice: what have we learned, and where do we go from here? Circ. Res. 89, 944–956. https://doi.org/10.1161/hh2301.100349.
Phan, D.T.T., Wang, X., Craver, B.M., Sobrino, A., Zhao, D., Chen, J.C., Lee, L.Y.N., George, S.C., Lee, A.P., Hughes, C.C.W., 2017. A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. Lab Chip 17, 511–520. https://doi.org/10.1039/C6LC01422D.
Poller, W., Hajjar, R., Schultheiss, H.P., Fechner, H., 2010. Cardiac-targeted delivery of regulatory RNA molecules and genes for the treatment of heart failure. Cardiovasc. Res. https://doi.org/10.1093/cvr/cvq056.
Radisic, M., Park, H., Shing, H., Consi, T., Schoen, F.J., Langer, R., Freed, L.E., Vunjak-Novakovic, G., 2004. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. Proc. Natl. Acad. Sci. Unit. States Am. 101, 18129–18134. https://doi.org/10.1073/pnas.0407817101.
Robertson, C., Tran, D.D., George, S.C., 2013. Concise review: maturation phases of human pluripotent stem cell-derived cardiomyocytes. Stem Cell. https://doi.org/10.1002/stem.1331.
Simpson, D.G., Sharp, W.W., Borg, T.K., Price, R.L., Terracio, L., Samarel, A.M., 1996. Mechanical regulation of cardiac myocyte protein turnover and myofibrillar structure. Am. J. Physiol. Cell Physiol. 270 (4), C1075–C1087.
Somasuntharam, I., Boopathy, A.V., Khan, R.S., Martinez, M.D., Brown, M.E., Murthy, N., Davis, M.E., 2013. Delivery of Nox2-NADPH oxidase siRNA with polyketal nanoparticles for improving cardiac function following myocardial infarction. Biomaterials 34, 7790–7798. https://doi.org/10.1016/j.biomaterials.2013.06.051.
Suckau, L., Fechner, H., Chemaly, E., Krohn, S., Hadri, L., Kockskamper, J., Westermann, D., Bisping, E., Ly, H., Wang, X., Kawase, Y., Chen, J., Liang, L., Sipo, I., Vetter, R., Weger, S., Kurreck, J., Erdmann, V., Tschope, C., Pieske, B., Lebeche, D., Schultheiss, H.P., Hajjar, R.J., Poller, W.C., 2009. Long-term cardiac-targeted RNA interference for the treatment of heart failure restores cardiac function and reduces pathological hypertrophy. Circulation 119, 1241–1252. https://doi.org/10.1161/CIRCULATIONAHA.108.783852.
Swaminathan, P.D., Purohit, A., Soni, S., Voigt, N., Singh, M.V., Glukhov, A.V., Gao, Z., He, B.J., Luczak, E.D., Joiner, M.L.A., Kutschke, W., Yang, J., Donahue, J.K., Weiss, R.M., Grumbach, I.M., Ogawa, M., Chen, P.S., Efimov, I., Dobrev, D., Mohler, P.J., Hund, T.J., Anderson, M.E., 2011. Oxidized CaMKII causes cardiac sinus node dysfunction in mice. J. Clin. Invest. 121, 3277–3288. https://doi.org/10.1172/JCI57833.
The FANTOM Consortium and the RIKEN PMI and CLST (dgt), 2014. A promoter-level mammalian expression atlas. Nature 507, 462–470. https://doi.org/10.1038/nature13182.
Thomas, C.E., Ehrhardt, A., Kay, M.A., 2003. Progress and problems with the use of viral vectors for gene therapy. Nat. Rev. Genet. 4, 346–358. https://doi.org/10.1038/nrg1066.
Voets, T., Droogmans, G., Wissenbach, U., Janssens, A., Flockerzi, V., Nilius, B., 2004. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430, 748–754. https://doi.org/10.1038/nature02732.

Please cite this article in press as: Qiao, Y., et al., Multiparametric slice culture platform for the investigation of human cardiac tissue physiology, Progress in Biophysics and Molecular Biology (2018), https://doi.org/10.1016/j.pbiomolbio.2018.06.001